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Comparison of mass-transfer and isotopic dilution methods for estimating milk intake in Antarctic fur seal pups

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Abstract The efficacy of a new mass-transfer method for estimating milk intake was examined in Antarctic fur seals (*Arctocephalus gazella*) at Iles Kerguelen. Our method differed from previous mass-transfer approaches in that we estimated milk-mass transfer as the maternal mass lost (MML; kg) during an attendance bout, less the mass lost to metabolic maintenance (MML_E) over that time. MML was significantly related to pup mass-gain (PMG) and attendance bout duration (*d* days) as follows: $MML = 1.106PMG + 1.002d$ ($r^2 = 0.998$). Based on this and previous studies, we estimated that the MML_E was 0.0285 kg kg^{-1} day^{-1} for lactating females; and we developed the following milk-mass transfer equation: $MML_M = 1.106PMG + 1.002d - 0.0285MM \quad d$ (where MM is maternal mass). Milk-mass intake was also estimated in an additional 21 pups, using the isotopic dilution method. These values were then compared with estimates based on the milk mass-transfer equation for the same individual pups. A pair-wise comparison indicated that milk-mass transfer estimated using tritium dilution methods were significantly lower than those based on mass-transfer (MML_M). Furthermore, the absolute PMG exceeded tritium dilution estimates of milk-mass transfer in 35% of cases. In contrast, all milk-mass transfer estimates using the mass transfer method were greater than PMG. Overestimation of metabolic water production (MWP), leading to a smaller proportion of the total water intake being attributed to milk ingestion, is believed to be the most likely cause for significant underestimation of milk-mass transfer using the tritium dilution method. Consumption of exogenous water by pups is the most likely reason for the overestimation of MWP, although errors in estimated milk water content may have also contributed to underestimates. We conclude that, in our study, the mass-transfer method provided a more reliable estimate of milk-mass transfer than the isotopic dilution method; and we argue that, under certain conditions, it provides a practical alternative method where the assumptions of isotopic dilution methodology (e.g., all exogenous water from maternal milk) and quantitative parameters (e.g., maternal milk water content) may either be violated or impractical to measure.

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Introduction

Estimating the milk intake of otariid seals (fur seals, sea lions) has received much attention in recent years, primarily in studies aiming to examine the quantity and quality of milk delivered to pups during attendance bouts and how these vary in relation to the duration of the preceding foraging trip, the foraging behaviour of the mother and the sex, mass and age of the pup (Arnould et al. 1996a, 2001a; Arnould and Hindell 2002; Costa and Gentry 1986; Donohue et al. 2002; Higgins et al. 1988; Lea et al. 2002; Oftedal et al. 1987; Robinson 2002). In a few species, milk intake has been estimated over longer periods in order to gain estimates of the average milk intake during different parts of lactation and to examine variation within and between species and sex and between seasons of contrasting prey resources (Arnould et al. 1996a; Arnould and Hindell 2002; Costa and Gentry 1986; Donohue et al. 2002; Oftedal et al. 1987).

Estimating the total investment or transfer of milk from mother to pup throughout lactation has proved very difficult in otariids, due to the duration of lactation, the large number of alternating foraging trips and shore attendance bouts, the absence of robust methods for quantitatively measuring energy transfer/milk intake over long time periods and the isolated locations of many populations. As a consequence, in the otariid seals that have been studied, energy transfer has been estimated only for certain portions of the total lactation period (Costa 1991). In phocids, in comparison, due their relatively short and primarily "fasting" lactation strategy, measures of energy and mass transfer can be readily estimated. As a consequence, the total investment of energy during lactation has been measured for a number of species (Stewart and Lavigne 1984; Fedak and Anderson 1987; Anderson and Fedak 1987; Bowen et al. 2001; Tedman and Green 1987; Ortiz et al. 1984; Costa et al. 1986).

In otariids, the most common approach for estimating milk intake has been the isotopic dilution method using either tritiated or deuterated water (Arnould et al. 1996a, 2001a; Arnould and Hindell 2002; Costa and Gentry 1986; Donohue et al. 2002; Higgins et al. 1988; Lea et al. 2002; Oftedal and Iverson 1987; Oftedal et al. 1987; Robinson 2002). There is a range of isotopic dilution methods used, all requiring the measurement of total water influx, but varying in the way that metabolic water production (MWP) is estimated. These include utilizing indirect colorimetry to estimate MWP (Higgins et al. 1988; Oftedal et al. 1987) and estimating MWP from water influx during fasting periods, using a hydrogen isotope (deuterium or tritium; Arnould and Boyd 1995; Costa and Gentry 1986; Donohue et al. 2002). There is also the two-isotope method that utilizes both tritiated and deuterated water and does not require estimation of MWP (Holleman et al. 1975, 1988). In this method, one isotope is given to the mother and the other to the pup; and milk intake is then estimated from the isotope ingested from the mother. Another two-isotope method measures the concurrent turnover rates of a hydrogen isotope and an oxygen isotope (O^{18}) to estimate MWP. Both the hydrogen and oxygen isotopes decline as a function of water influx. However, the O^{18} also declines as a function of CO_2 production and the difference can be used to estimate MWP. There are a number of disadvantages with these isotope methods, including the relatively short period over which water turnover measures can be made (10–20 days for typical doses), such that milk intake is typically averaged over a time period that may include multiple suckling bouts and (with the exception of the two-isotope methods) pups are required to obtain all their water from their mother's milk, with no exogenous water being consumed (Costa 1991). Although the latter is usually assumed in such studies to either not occur or be negligible, reports of pups drinking water (either fresh or sea) are not uncommon (Gentry 1981; Lea et al. 2002). A recent study on Antarctic fur seal pups showed that consumption of water (salt or fresh) may account for up to 50% of estimated MWP, equivalent to the ingestion of 1 l of water every 4 days by a 10-kg pup (Lea et al. 2002).

The transfer of mass to pups during attendance bouts has also been used as an index of milk intake in otariid pups (Georges and Guinet 2000; Goldsworthy 1995; Guinet and Georges 2000; Guinet et al. 1999, 2000) and, although intuitive, it does not provide a direct quantitative measure of milk-mass transferred. The major disadvantage of this method is that the gain in mass by pups through ingestion of milk is a composite of the mass gained through milk intake and the mass lost as a consequence of maintenance metabolism and assimilation efficiency (including excretion).

Here, we examine the potential application of a mass-transfer approach to estimate milk-mass transfer in otariids. This method differs from previous techniques in that it aims to estimate maternal mass lost (MML) during an attendance bout, using the gain in mass by the pup and the duration of the attendance bout. Given that lactating females are only losing mass due to milk transfer and maintenance metabolism (including excretion) during attendance bouts, the difference between the total mass lost by a female and that lost to metabolic maintenance should equal the total milk-mass transferred to the pup. Our approach here is to develop a mass-transfer method in one group of pups and then apply it to an independent group of pups where milk intake has also been estimated using the most commonly used isotopic dilution method, where MWP is estimated from the water influx of fasting pups. We discuss the efficacy of both techniques.

Materials and methods

Study site

This study was conducted on a colony of Antarctic fur seals at Cap Noir, Courbet Peninsula (49°07′S, 70°45′E), Iles Kerguelen (Southern Indian Ocean) between 4 February and 7 March 1998. The colony consisted of approximately 775 mother–pup pairs (unpublished data).

Milk consumption—maternal mass-transfer

During the course of the study, 13 lactating females returning from foraging trips were captured upon reuniting with their pup. Both mothers and pups were weighed prior to the commencement of nursing, using

50×0.2 kg and 20×0.1 kg spring balances, respectively. The standard length (nose to tail tip) of mothers was also measured to the nearest centimetre. Mothers were captured using a hoop-net and marked with sequential numbers, using hydrogen peroxide (Clairol Borne Blonde; Bristol–Myers Squibb, Ryde, Australia). Pups were identified using individually numbered plastic tags (size 1 Super tags; Dalton Supplies, Henley-on-Thames, UK) applied to the trailing edge of the fore-flippers. These were removed at the end of the study period. Mother–pup pairs were then left to nurse uninterrupted, until recapture, when they were reweighed. Mother–pup pairs were usually left to nurse for at least 1 day (range 0.9–2.8 days), after which they were opportunistically captured for reweighing. The time between the initial and final capture and weighing of each mother–pup pair was recorded.

Milk consumption—isotopic dilution

Milk consumption was estimated for 21 pups during a single maternal attendance bout, using the hydrogen isotope (HTO) dilution method (Costa 1987). Pups that had been fasting for at least 3 days since the previous suckling bout were captured, weighed with a 20×0.1 kg spring balance and sexed. Individual pups were identified by flipper tags (as above). Pups received an intramuscular injection (1 ml) of pre-weighed HTO (200 μCi HTO ml^{-1}) stored in Wheaton vials (Milleville, N.J., USA). After the contents of the vial had been injected, it was rinsed twice with about 1 ml of sterile saline, which was then injected to ensure delivery of the complete dose.

Pups were subsequently held in an enclosure for 3 h to allow isotopic equilibration (Arnould et al. 1996a; Costa 1987), after which time an equilibration blood sample (E_1) was taken to determine initial total body water (TBW_i; kg) and the pups were released. Pups were recaptured and re-weighed every 2 days following the initial capture, prior to the return of their mothers. At this time, additional blood samples (E_2, E_3, etc.) were taken. Two days after the subsequent maternal attendance bout, pups were recaptured and reweighed; and the procedure for estimating TBW_i was repeated, using a lower concentration of HTO (1 ml 50 μCi HTO ml^{-1}). Pups were released after 3 h, following the final equilibration (E_f) blood sample. Blood samples were left to stand for several hours before centrifugation, after which the plasma fraction was pipetted into plastic tubes and frozen at −20°C until analysis.

Samples were thawed and three replicate 0.1-ml aliquots were distilled, following the methods of Ortiz et al. (1978) . The volume of distilled water was then measured and 3.5 ml of scintillation cocktail (EcoLite ICN; Costa Mesa, Calif., USA) was then added to each vial. Replicates were counted for 10 min in a Beckman LS6500 multipurpose scintillation counter.

Total body weight was calculated from the dilution space and corrected using the equation from Arnould et al. (1996b): $TBW = 0.11 + 0.97HTO$. Milk water intake (MWI) was calculated using the modified equation from Ortiz et al. (1984):

$$MWI = \frac{TWI - MWP}{milkH_2O},$$

where TWI is total water influx, MWP (ml kg^{-1} day^{-1}) is metabolic water production and milkH_2O is the proportional milk water content. TWI was calculated by estimating the decrease in specific activity of the body water, using Nagy and Costa's (1980) equations 5 and 6 to calculate rates of water turnover. MWP was calculated from the TWI of fasting pups. Where values for individual pups could not be derived (six out of 21 cases where nursing commenced before additional equilibration samples were obtained), the average MWP was used. Based on the milk lipid to milk water conversion equation of Arnould and Boyd (1995), the milk water content of sampled fur seal milk from Cap Noir was estimated to be 41.2% (SD = 8.87, n = 21; M.A. Lea, unpublished data). A regression equation was developed to convert milk volume to milk mass by multiplying the proportions of milk water, lipid and protein (following Goldsworthy and Crowley 1999) by their respective specific gravities (SG): water SG = 1, lipid SG = 0.89 (the main milk lipid is oleic acid) and protein SG = 1.35 (the main milk protein is casein; Oftedal et al. 1987). Based on these values, the regression to convert percentage milk water content (mwc) to milk SG ($milk_{SG}$) was $milk_{SG} = 1.059 - 0.001mwc$. Following this, milk transfer estimates based on tritium were converted from litres to kilograms by multiplying by 1.02, assuming a milk water content of 41.2% (SD = 8.87, n = 22, M.A. Lea, unpublished data).

Pups involved in the isotope dilution experiment were captured and weighed regularly within 1 day of the arrival and departure of their mother. Individual regressions of pup mass loss per day were calculated during the fasting period of each pup in order to calculate their mass at the time of the maternal arrival ashore. Guinet et al. (2000) showed that the daily mass loss of fasting pups differs according to their sex, mass and the duration of the fasting period. As such, when not measured directly, pup mass at the maternal arrival and departure were estimated using the equations from Guinet et al. (2000), which were derived using pups from the same site and season. Maternal attendance was measured by direct observation, based on twice daily checks of the colony between 0800–0900 h and 1800–1900 h. Exact arrival and departure times were recorded where possible, otherwise the median arrival and departure times, as determined from 20 additional females fitted with VHF transmitters (in the same season), were used (see Guinet et al. 2000). Maternal mass and standard length were recorded for the mothers of experimental pups as above and, where several masses and lengths had been recorded, the mean of these was used. Statistical analyses were undertaken using SYSTAT ver. 10 (SPSS, Chicago, Ill., USA). Statistical significance was reported at $P < 0.05$, unless otherwise stated.

Results

Estimates of MML during attendance bouts

A backwards stepwise general linear model with MML as the dependent variable and with pup mass gain (PMG), the period (d days) between weighings and the initial maternal mass (MM) and maternal length (ML) as independent variables indicated that MML was significantly related to both PMG and d ($F = 2636.846$, $P < 0.001$, $r^2 = 0.998$, $n = 13$) as follows:

$$\text{MML (kg)} = 1.106\,\text{PMG} + 1.002d \quad (1)$$

Pup sex was not included as an independent variable, due to the limited numbers of mothers with female pups in our sample.

Estimating components of MML

While nursing pups, MML is attributable to mass lost to maintenance metabolism (MML_E; including excretion) and milk transfer (MML_M); i.e., $MML = MML_E + MML_M$. In order to estimate MML_M, it is necessary to subtract MML_E from MML. The approach used to estimate MML_E is detailed below.

The average mass-specific mass-loss (MSML) rate of nursing females during attendance bouts was 0.093 kg kg^{-1} day^{-1} (SD = 0.024, $n = 13$; Table 1). The MSML rate is equivalent to $[(MML_E + MML_M)/\text{initial MM}]/d$. Assuming that $PMG < MML_M$, then (based on our data) the maximum $MML_E = MML - PMG$, or 0.038 kg kg^{-1} day^{-1} (SD = 0.006, $n = 13$; Table 1). Figure 1 shows the relationship between variance in the estimated rate of daily MML_E and the percentage of cases in our study where $PMG > MML_M$. The figure indicates that no estimates of PMG exceed MML_M until the daily MML_E rates exceed 0.030 kg kg^{-1} day^{-1}, suggesting that the actual rates of MML_E for lactating females are somewhat lower than this figure. Based on the daily mass-loss rates of two fasting non-lactating females and seven male *Arctocephalus gazella* at South Georgia, Costa and Trillmich (1988) estimated that non-lactating *A. gazella* lost 0.0237 kg kg^{-1} day^{-1} (SD = 0.005, range 0.018–0.032). These results suggest that the actual daily MML_E rates in fasting lactating females lie between the estimates of Costa and Trillmich (1988) and our estimated maximum value of 0.030 kg kg^{-1} day^{-1}. Oftedal (1985) and Oftedal and Gittleman (1989) calculated that the theoretical resting metabolic rates of lactating herbivores and carnivores was 1.2-fold that of non-lactating mammals. Accordingly, the Costa and Trillmich (1988) figure is adjusted to 0.0285 kg kg^{-1} day^{-1} for lactating females, approximately 5% less than our theoretical maximum MML_E rate. Using this estimate of MML_E, 67% (SD = 7.7%, range 54.0–80.9%) of MML during nursing bouts is attributable to milk-mass transfer (MML_M).

Equation 1 can therefore be modified to estimate MML_M as follows:

$$MML_M(1)\ \text{kg} = 1.106\,\text{PMG} + 1.002d - 0.0285\,\text{MM}d \quad (2)$$

where MM is the maternal mass (kg) and d is the duration of the attendance bout, in days.

Table 1 Details of *A. gazella* mother–pup pairs used in the mass-transfer study including MM, ML, time between initial and subsequent weighing, pup sex, MML, PMG, MML-PMG, MSML rate, the maximum MML to maintenance metabolism (MML_E) and the estimated milk mass (MML_M) transferred, using Eq. 2

Identity number	MM (kg)	ML (cm)	Time (days)	Pup sex	MML (kg)	PMG (kg)	MML-PMG (kg)	MSML (kg.kg^{-1}.day^{-1})	Max MML_E (kg kg^{-1} day^{-1})	Estimated milk transfer MML_M (kg)
94	34.5	113.0	1.8	M	6.2	4.0	2.2	0.102	0.036	4.46
2	27.8	112.0	0.9	M	3.0	1.8	1.2	0.119	0.047	2.18
18	33.4	122.0	0.9	F	3.2	1.9	1.3	0.106	0.043	2.15
36	32.3	116.5	1.0	M	4.8	3.3	1.5	0.149	0.047	3.73
23	33.2	119.0	2.0	M	4.5	2.0	2.5	0.068	0.038	2.32
29	24.9	105.0	1.2	F	3.0	1.8	1.2	0.100	0.040	2.34
50	37.6	131.0	1.0	M	3.4	2.1	1.3	0.089	0.034	2.25
35	29.6	113.0	1.0	F	2.7	1.7	1.0	0.090	0.034	1.98
55	29.1	108.0	1.8	M	5.0	2.7	2.3	0.098	0.045	3.29
B5	28.7	112.0	1.2	M	2.4	1.0	1.4	0.069	0.040	1.33
62	38.5	125.0	1.9	M	4.5	2.2	2.3	0.062	0.031	2.28
112	33.2	117.5	2.0	F	5.1	3.1	2.0	0.078	0.031	3.54
70	31.9	113.0	2.8	M	6.9	4.0	3.0	0.077	0.033	4.63
Mean	31.9	115.9	1.5		4.2	2.27	2.4	0.093	0.038	2.81
SD	3.9	7.1	0.6		1.4	0.78	0.9	0.024	0.006	1.02

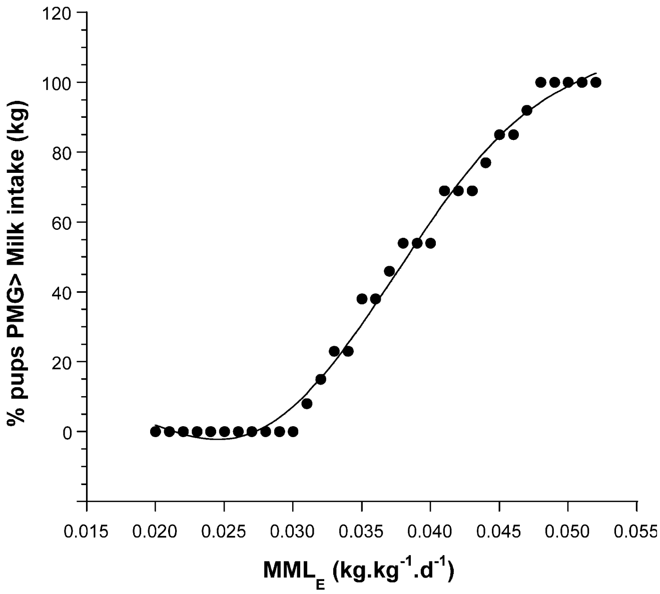

Fig. 1 The effect of changing values of the daily MML_E of lactating *A. gazella* females and the percentage of estimates of milk-mass transfer based on Eq. 2 where PMG exceeded the estimated milk intake. Results indicate that the actual fasting MML_E rates are likely to be less than 0.030 kg kg^{-1} day^{-1}. Each point represents an increment of 0.001 kg kg^{-1} day^{-1} MML_E. *d* Day

Sensitivity analysis

To use the mass-transfer method for estimating milk intake during attendance bouts, PMG and time (*d*) values are necessary. If these are measured accurately, then most of the error in milk transfer estimates is due to uncertainty in MM at the time of haul-out (unless directly measured) and MML_E. For the 13 mother-pup pairs in this study, a ±10% variation in the estimated MM resulted in <5% variation in estimates of milk transfer (0.14 kg, 4.9%). Similarly, a ±10% change in the daily rate of MML_E resulted in <5% variation in the estimated milk transfer (0.14 kg, or 4.8% of estimated milk intake). Further, the standard deviation of 0.005 kg kg^{-1} day^{-1} reported for the nine fasting (non-lactating) *A. gazella* in Costa and Trillmich's (1988) study amounts to a standard deviation of only ±0.024 kg estimated milk-mass transfer, based on results from our study animals.

Based on the estimated milk mass transfer of the 13 pups, assimilation efficiency (PMG/MML_M) at the time of final weighing was estimated to be 86.1% (SD = 5.9, range 75.2–96.5). There was no relationship between the duration of maternal attendance (time) and estimated assimilation efficiency of pups ($F_{1,2} = 0.345$, $P = 0.569$).

Estimating milk intake using tritiated water

Milk consumption rates of a separate group of 21 pups were estimated for a single maternal attendance bout, using the hydrogen isotope dilution technique (Table 2). There was a strong positive relationship between body mass and the TBW of male pups (TBW = 0.632 × mass, $r^2 = 0.997$) and female pups (TBW = 0.997 + 0.501 × mass, $r^2 = 0.987$); and the slopes of these regressions were different for each sex (ANCOVA; $F_{1,18} = 4.151$, $P = 0.054$). MWP was calculated for 15 of 21 pups (mean = 31.97 ml kg^{-1} day^{-1}, SD = 5.07; Table 2). Initial measurements of MWP were taken when pups had been fasting for an average of 5.9 days (range 3–8 days), with up to four fasting estimates of MWP being made for each pup (Table 2). There was no significant relationship between MWP, sex and initial mass of the pup (ANCOVA; mass $F_{1,11} = 2.550$, $P = 0.139$; sex $F_{1,11} = 0.015$, $P = 0.916$).

For the same 21 pups, body mass immediately prior to and post attendance bouts were used to calculate PMG. In conjunction with data on attendance bout duration (*d*), the MML_M was estimated using Eq. 2. Estimates of milk-mass transfer based on tritium were significantly lower than those based on MML_M (paired *t*-test: $t = 2.835$, df = 17, $P = 0.011$; Table 2).

Estimates of milk-mass transfer based on the tritiated water dilution method were less than the PMG over the same attendance bout in seven of the 21 pups (mean = −0.021 kg < PMG, SD = 0.889, $n = 21$), indicating that the tritium method underestimated milk-mass transfer in 35% of cases. In contrast, all milk-mass transfer estimates using the MML_M method were greater than PMG (mean = 0.678 kg > PMG, SD = 0.310, $n = 18$; Table 2). Despite these differences, milk intake estimates based on the tritiated water dilution technique were highly correlated with those based on MML_M (Pearson correlation statistic = 0.844, $P < 0.001$, with two outliers deleted; Fig. 2) and the

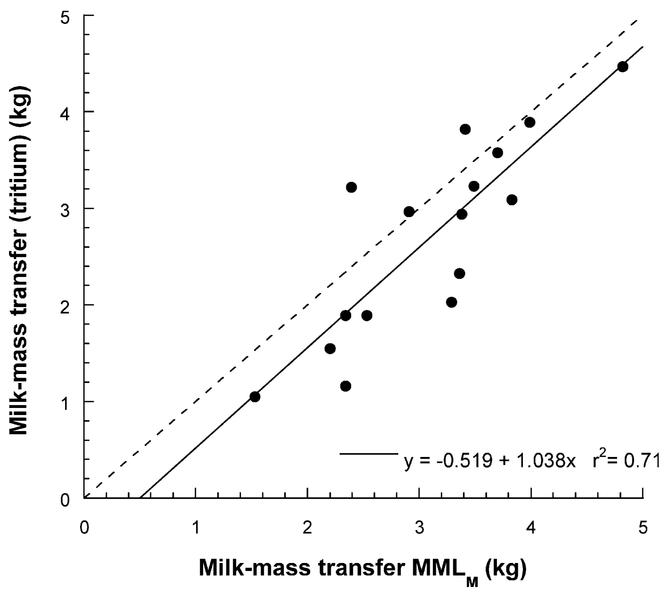

Fig. 2 The relationship between milk-mass consumed during attendance bouts, based on estimates from MML_M and the tritium dilution method in *A. gazella* pups at Iles Kerguelen. *The dotted line* indicates parity in results from both techniques

Table 2 Comparison of milk-mass transfer estimated from tritium/isotopic dilution and mass-transfer method in *A. gazelle*

			Estimated milk intake, tritium dilution method						Estimated milk intake, mass-transfer method				Estimated milk intake (kg)	
Pup number	Sex	Initial mass (kg)	TBW (l)	Mass change (kg)[a]	MWP (l kg^{-1} day^{-1}) (D, n)[b]	MWI l kg^{-1} d^{-1}	Days to calculate MWI	Estimated milk consumption (l)	Previous FTD[d] (days)	Shore (days)	Estimated PMG (kg)	MM (kg)	Tritium	MML_M
4	M	6.90	4.99	2.60	0.021 (7, 2)	0.120	3.98	3.38	11.44	1.56	3.15	–	3.45	
6	M	12.10	7.30	1.50	–	0.029	3.12	1.14	8.44	0.09	2.10	28.2	1.16	2.34
12	F	10.00	5.80	3.18	0.029 (5, 3)	0.111	4.77	3.81	16.89	2.35	3.50	33.37	3.89	3.99
18	F	8.90	5.28	1.90	–	0.069	6.88	1.86	11.5	2.48	1.90	31.78	1.89	2.34
24	M	7.70	4.86	2.04	0.030 (8, 1)	0.067	5.91	2.88	10.95	2.05	2.60	26.6	2.94	3.38
25	M	6.95	4.56	0.65	0.035 (-, 2)	0.039	3.76	1.03	7.14	1.56	1.00	25.7	1.05	1.53
31	F	5.15	4.55	0.90	0.028 (8, 1)	0.066	7.89	1.52	15.14	1.86	1.40	22.8	1.55	2.2
32	M	6.60	3.55	2.00	–	0.053	6.83	1.65	13.49	2.35	3.00	23.9	1.69	4.07
39	M	11.30	6.89	1.80	0.035 (5, 2)	0.055	4.93	3.32	7.65	2.05	2.80	–	3.38	
41	M	10.70	7.15	1.10	0.028 (4,2)	0.070	6.05	3.03	10.29	1.8	3.00	25.15	3.09	3.83
45	F	5.50	3.74	1.90	–	0.110		3.16	12.14	2.56	2.30	22.2	3.23	3.49
53	M	8.25	5.19	2.65	0.028 (7, 3)	0.108	3.89	3.51	14.78	1.22	3.30	33.6	3.58	3.7
58	F	7.35	4.71	1.40	–	0.109	3.71	3.16	9.44	1.25	1.85	25.4	3.22	2.39
60	F	10.40	6.08	1.45	0.032 (7, 2)	0.054	5.05	2.91	10.44	1.04	2.74	39.15	2.97	2.91
63	F	9.70	5.87	2.45	0.040 (5, 3)	0.103	5.14	3.74	11.58	1.67	2.85	29.75	3.82	3.41
70	M	9.70	6.09	2.90	0.039 (5, 3)	0.021	15.05[c]	1.09	17.98	4	3.75	27.8	1.12	4.99
79	F	5.20	3.62	1.90	–	0.088	4.88	2.28	8.95	1.84	2.50	23.9	2.33	3.36
84	F	7.45	3.66	1.95	0.031 (3, 2)	0.072	3.87	1.71	13.44	1.56	2.60	–	1.75	
86	F	9.40	5.52	2.05	0.039 (7, 1)	0.069	4.04	1.99	7.68	1.23	2.90	32.8	2.03	3.29
94	M	8.45	5.38	3.47	0.034 (6, 4)	0.100	5.9	4.38	10.08	3.04	4.06	31.4	4.47	4.82
95	F	7.40	4.80	1.40	0.030 (5, 3)	0.083	3.72	1.85	10.71	2.05	1.60	22.2	1.89	2.53
Mean		8.34	5.22	1.96	0.032	0.076	5.49	2.54	11.44	1.89	2.61	28.09	2.59	3.25
SD		1.99	1.11	0.72	0.005	0.029	2.59	1.00	2.98	0.8	0.77	4.77	1.02	0.92

[a]Mass change between equilibration samples
[b]*D* number of days fasting prior to first equilibration, *n* number of pre-feed equilibrations used to estimate MWP
[c]Mother of pup 70 on long foraging trip
[d]*FTD* foraging trip duration

slope did not differ significantly from a slope of one (ANCOVA testing homogeneity of slopes, $F_{1,28} = 0.046$, $P = 0.832$).

Discussion

This study shows a very high correlation between MML during attendance bouts, PMG and the duration of maternal attendance. As such, the mass-transfer method provides an appropriate index of milk transfer from mother to pup during attendance bouts. Similarly, Georges and Guinet (2000) also found a significant correlation ($r^2 = 0.832$) between MML, PMG, ML and attendance bout duration in subantarctic fur seals (*A. tropicalis*) at Amsterdam Island. The extent to which such indices can be used as direct measures of milk-mass transfer from mother to pup is dependent on MM data and the daily MSML rate attributable to the fasting metabolic rate. If these are known, then the method provides an alternative to the isotopic dilution method for estimating milk intake in otariid seals.

Although this study cannot validate the accuracy of the mass-transfer method for estimating milk intake, our results show a strong correlation between estimates of milk intake based on mass-transfer and tritium dilution methods, indicating that it is a reliable method for estimating milk transfer. Further, our results suggest that the mass-transfer method may provide a more reliable estimate of milk transfer, as estimates based on the tritium dilution method underestimated milk-mass transfer in at least 35% of cases, compared with 0% using the mass-transfer method, assuming that PMG during maternal attendance cannot exceed the milk-mass transferred.

The underestimation of milk intake using the tritium dilution method is most likely due to overestimates of MWP, leading to a smaller proportion of the TWI being attributed to milk ingestion. Estimates of MWP for pups in our study, based on fasting water influx rates, are among the highest reported for otariid seal pups. Although our estimates of fasting water influx are very close to those reported by Lea et al. (2002; 0.033 kg kg^{-1} day^{-1}) for free-ranging *A. gazella* pups at the same study site, they are almost double those reported for free-ranging post-moult *Callorhinus ursinus* pups [0.061–0.0179 kg kg^{-1} day^{-1} (Donohue et al. 2002), 0.0208 kg kg^{-1} day^{-1} (Costa and Gentry 1986)] and are slightly higher than those reported for free-ranging *A. gazella* pups at South Georgia (0.0265 kg kg^{-1} day^{-1} males, 0.0284 kg kg^{-1} day^{-1} females; Arnould et al. 1996a). An overestimation of MWP based on fasting water influx may be attributable to the different activity rates of fasting and nursing pups. Studies have shown that pups held in enclosures have lower rates of MWP, compared with free-ranging pups (Arnould et al. 1996a, 2001b; Donohue et al. 2000; Lea et al. 2002) and, as a consequence, fasting water influx rates may overestimate MWP during nursing bouts. However, Lea et al. (2002) found no difference in the MSML rates of free-ranging and enclosed pups, suggesting that differences between the fasting water influx of the two groups were not a consequence of differences in activity and MWP.

The most likely explanation for overestimating MWP was due to the consumption of exogenous water by pups. Lea et al. (2002) demonstrated this in their experimental study on the potential importance of water drinking in *A. gazella* pups at Iles Kerguelen. The study found that pups held in open-air enclosures for 4 days without access to water had significantly lower TWI, compared with enclosed pups fed 300 ml of water and free-ranging pups not fed water over the same duration (Lea et al. 2002). The additional water ingested was equivalent to about 250 ml day^{-1} for a 10 kg pup, resulting in considerable underestimation of milk water flux. This is consistent with results from this study, which also suggest a significant underestimation of milk transfer using the tritium dilution method. Results from our study and that of Lea et al. (2002) bring into question the appropriateness of the isotopic dilution method for estimating milk intake at locations where the drinking of exogenous water (or milk-stealing) could account for a significant proportion of the TWI of pups. At such sites, the two-isotope method would be the only isotopic dilution approach that could account for exogenous water.

Another possible reason why milk transfer estimates based on the tritium dilution method underestimated milk transfer may be due to using an average value of milk water content fed to pups. Given that milk content can vary considerably among otariid females, throughout lactation and during the course of an attendance bout (Costa and Gentry 1986; Oftedal et al. 1987; Arnould and Boyd 1995; Gales et al. 1996; Arnould and Hindell 2002; Goldsworthy and Crowley 1999; Georges et al. 2001), errors in milk water content may have resulted in an underestimation of milk intake. Based on results from pups injected with tritium in our study, the effect on changing milk composition (% mwc) on the estimated milk consumption followed this equation:

$$\text{milk intake (kg)} = 119.04\,\text{mwc}^{-1.030}.$$

As such, for the mean milk mass transfer among the tritium pups sampled to be equal to those estimated by mass transfer, the milk water content would have to be reduced to 33.13%, approximately 8% (close to 1 SD) less than the mean of the milk samples collected at our study site (M.A. Lea, unpublished data). A mwc ±1 SD would have produced estimates of milk-mass transfer based on tritium dilution that were −1.103 kg less and 0.083 kg more than those estimated using mass-transfer methods, respectively.

Previous studies have used the PMG over maternal attendance bouts as an index of milk-mass transfer

(Goldsworthy 1995; Guinet et al. 1999, 2000). However, PMG represents a composite of the mass gained due to milk intake and the mass lost through metabolic costs and assimilation efficiency. It is difficult to estimate the metabolic MSML rate of pups during nursing bouts, although they are likely to be greater than in pups that have been fasting for more than 2 days (Guinet et al. 2000). Also, there is little quantitative data on the assimilation efficiency of otariid pups. In comparison with pups, lactating female otariids only lose mass during attendance bouts, as a consequence of MML_M and MML_E. Hence, MML provides a more tractable part of the mass-transfer equation by which to estimate milk transfer; and, in theory, as long as MML_E can be estimated, the remaining mass loss should be attributable to MML_M.

In our study, the MSML rates of lactating females (0.093 kg kg^{-1} day^{-1}) were considerably higher (almost 3-fold) than that reported for lactating *A. gazella* females at South Georgia (0.0315 kg kg^{-1} day^{-1}; Costa and Trillmich 1988). However, the latter study was undertaken on females during the perinatal period, suggesting that milk transfer rates are very much lower during this period than during subsequent attendance bouts.

The mass-transfer method could be used to estimate milk intake in otariids over extended periods and possibly throughout the entire duration of lactation, requiring estimates of mass-transfer per attendance bout and their duration. By monitoring the mass of pups during fasting bouts, with a good knowledge of maternal haul-out and departure times, pup MSML rates can be used to estimate pre- and post-attendance bout mass and PMG (see Guinet et al. 1999, 2000; Georges and Guinet 2000); and from these MML and milk-mass transfer can be estimated. An estimate of maternal mass at haul-out is required in order to estimate the component of MML due to maintenance metabolism and excretion (MML_E).

Because the mass-transfer method indirectly estimates the milk-mass transferred, its accuracy is dependent upon the precision of measures of MM, PMG, attendance duration and estimates of the maintenance MSML rate. The ease of obtaining such data is clearly dependent on the species and study site; and the precision of the method would benefit from more data on the MSML rates of fasting lactating females. Although our study has identified that mass-transfer is an appropriate measure of milk-intake and under certain circumstances has advantages over isotopic dilution methods, true validation of the method is required. The most appropriate method to test its accuracy would be the two-isotope method.

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